AD NUMBER

AD481026

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; Feb 1966. Other requests shall be referred to United States Army Biological Center, Fort Detrick, MD.

AUTHORITY

BORL D/A ltr, 28 Sep 1971

TECHNICAL MANUSCRIPT 283

ANTIBODY FOR PSITTACOSIS GROUP AGENTS IN ASCITIC FLUIDS OF MICE IMPLANTED WITH SARCOMA 180

James T. Duff
Altha C. Mumford

FEBRUARY 1966

UNITED STATES ARMY
BIOLOGICAL CENTER
FORT DETRICK

DDC AVAILABILITY NOTICES

Qualified requestors may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

U.S. ARMY BIOLOGICAL CENTER
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 283

ANTIBODY FOR PSITTACOSIS GROUP AGENTS IN ASCITIC FLUIDS OF MICE IMPLANTED WITH SARCOMA 180

James T. Duff

Altha C. Mumford

Medical Investigation Department
MEDICAL SCIENCES LABORATORY

Project 1C622401A072

February 1966

In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

ABSTRACT

Preparation of neutralizing antisera to psittacosis group agents in laboratory animals is attended by practical difficulty, and antisera to these agents are available in limited quantity. Methods for the production of immune ascitic fluids in mice suggested a means of obtaining antisera for these agents. Mice were given three intraperitoneal injections of formalinized vaccines prepared from either the Borg or 6BC strains of the psittacosis group agents and challenged by the same route 3 weeks after the last injection. Sarcoma 180 cells were implanted in the surviving mice 2 weeks after challenge, and ascitic fluids (20 to 30 ml/mouse) were collected at 1- to 2-week intervals thereafter. Borg immune ascitic fluid obtained through day 29 neutralized greater than 100,000 $MIPLD_{50}$, and neutralizing activity was demonstrable up to 1:32. The neutralizing capacity of the 6BC immune ascitic fluid was much lower; approximately 1,000 to 10,000 $MIPLD_{50}$ were neutralized. Passive protection with Borg immune ascitic fluid was strongly evident on the 5th day following intraperitoneal immunization and had disappeared by the 10th day. Cross neutralization titrations on Borg immune ascitic fluid showed a low heterotypic titer for the 6BC agent. Neutralization was demonstrable by the intracranial route with fluids of high potency.

ANTIBODY FOR PSITTACOSIS GROUP AGENTS IN ASCITIC FLUIDS OF MICE IMPLANTED WITH SARCOMA 180

Protective neutralizing antibodies to the psittacosis group of microorganisms have been demonstrated in the sera of mice, roosters, and other laboratory animals by a number of investigators. Routine preparation of suitable antisera is attended by practical difficulty and, therefore, high-titer, homotypic neutralizing antisera for the various strains of this group of agents are available in very limited quantities, if at all available. The lack of suitable typing antisera has hindered investigators in classifying new isolates by serological techniques. Methods for the production of immune ascitic fluids in mice suggested a means of obtaining antisera for these agents. This paper describes a method whereby neutralizing antibody to two agents of the psittacosis group can be obtained from mice in considerable volume.

A strain of sarcoma 180 (S-180) obtained from Dr. Julius E. Officer, Jr., Fort Detrick, and maintained by routine implant passage in the Fort Detrick strain of white mice, was used in these studies. Formalinized vaccines for the Borg strain of the human pneumonitis agents and the 6BC strain of the psittacosis agent were utilized; procedures for the preparation of these vaccines have been described by Duff.*

For the production of antibody, mice were given three intraperitoneal (ip) injections of vaccine at weekly intervals and were challenged by the homologous route with infected egg yolk sac material 2 weeks after the last injection of vaccine. S-180 cells were implanted in the surviving mice 2 weeks after challenge, and ascitic fluids were collected at 1- to 2-week intervals following implantation. Approximately 20 to 30 ml of ascitic fluid was obtained from each mouse. The fluids were clarified first by low speed centrifugation and again by centrifugation at 10,000 rpm for 20 minutes. Neutralization titrations were carried out by the constant serum-varying virus method, using infected egg yolk sac suspensions of the Borg strain of the human pneumonitis agent or the 6BC strain of the psittacosis agent. The virus-serum mixtures were held 4 hours at room temperature and injected ip into 18- to 20-gram mice. The mice were examined daily and deaths were recorded over a 14-day period.

---

* Duff, J.T. 1965. An experimental psittacosis group vaccine (Borg Agent) prepared in a human diploid cell strain. Bacteriol. Proc. 118.
Duff, J.T. May 1965. Psittacosis group vaccine prepared in a human diploid cell strain, (Technical Manuscript 219). Medical Investigation Division, U.S. Army Biological Laboratories, Frederick, Maryland.

Neutralization titrations on four Borg immune ascitic fluids obtained over a period of 39 days, or four subsequent drainages, are summarized in Table 1. The time (in days) on which the different drainages were made following implantation with the sarcoma cells, is shown in the 2nd column. The various dilutions of Borg agent prepared and added to undiluted ascitic fluid are shown. The neutralization assays were conducted on different days, and the two respective controls are included in the table. Ascitic fluids obtained from mice that had not been immunized or challenged were used in the control assays. Ascitic fluids obtained during the first three drainages (or through 29 days) from the immune group of mice contained a high neutralizing capacity. Greater than 1,000,000 $MIPLD_{50}$ were neutralized with day 16 material, and greater than 100,000 $MIPLD_{50}$ were neutralized by ascitic fluids collected on days 22 and 29. By the 39th day, or the 4th drainage, the antibody level was considerably lower. No explanation can be given for the three survivors at the 10^{-2} dilution.

The dilution end point for the ascitic fluid obtained on the 2nd drainage is shown in Table 2. The final dilutions of immune ascitic fluid in the virus-fluid mixtures used in the neutralization assay were 1:2, 1:8, and 1:32. Normal ascitic fluid was used at a final dilution of 1:2 and 1:8. The data indicated that the immune ascitic fluid could be diluted considerably and still provide some neutralization. Immune ascitic fluid diluted 1:32 neutralized approximately 1 log of the Borg agent. Greater than 5 logs of the agent were neutralized when the immune ascitic fluid was used at a final dilution of 1:2.

The data presented in Table 3 show the duration of ip passive protection in mice obtained with undiluted immune ascitic fluid (F-18 of 4/13/65). Mice were given 0.5-ml amounts of immune ascitic fluid and challenged ip with various dilutions of infected yolk sac material of the Borg agent at 3 hours, 5 days, and 15 days following immunization. Passive protection was strongly evident on the 5th day and had disappeared by the 15th day, following administration of ascitic fluid.

A similar experiment on passive immunization that involved aerosol challenge was conducted, using a single challenge dose administered approximately 48 hours after immunization. Although 90% of the passively immunized mice died, the time to death was increased indicating some passive protection to aerosol challenge was afforded by the hyperimmune ascitic fluid.

TABLE 1. DURATION OF ANTIBODY IN BORG IMMUNE ASCITIC FLUIDS

Drainage Number	Day of Drainage	Neutralization Titration Data: Dilutions of Borg Agent (log_{10}) Added to Undiluted Ascitic Fluid								
		-1	-2	-3	-4	-5	-6	-7	-8	-9
1	16	4/5[a]	7/7	7/7	7/7	7/7	7/7			
2	22	3/4	4/4	4/4	4/4	4/4				
3	29	4/4	4/4	4/4	4/4	4/4				
4	39	0/4	3/4	0/4	0/4	0/4				
Normal Control (run with Drainage 1)			0/4	0/4	0/4	0/4	0/4	2/4	3/4	4/4
Normal Control (run with Drainage 2,3,4)					1/7	2/7	4/7	4/7	8/8	

a. Survivors/Totals.

TABLE 2. BORG IMMUNE ASCITIC FLUID DILUTION END POINT

Sample	Final Dilution of Ascitic Fluid	Calculated $MIPLD_{50}$/ml (log_{10})	Log Neutralization Index
Immune ascitic fluid[a]	1:2	1.3	>6.2
	1:8	4.3	3.6
	1:32	6.6	0.9-1.3
Normal ascitic fluid	1:2	7.5	
	1:8	7.9	

a. F-19 (4/27/65), second drainage.

TABLE 3. DURATION OF PASSIVE IMMUNITY (BORG IMMUNE ASCITIC FLUID)

Time of Challenge	Group	Challenge Titration Data: Dilution of Borg Agent (log_{10}) -1	-2	-3	-4	-5	-6	-7	-8	-9
24 hrs	Immune	1/3[a]		3/3		3/3		3/3		
	Control			0/3		1/3		3/3		3/3
5 day	Immune	2/3		3/3		3/3				
	Control			0/2		1/3		3/3		3/3
15 day	Immune	0/2	0/3	0/3	1/3					
	Control				0/2	0/3	0/3	2/3		

a. Survivors/Totals.

Investigations on the extent of serologic cross-over for the Borg immune ascitic fluid have been limited to the 6BC strain of the psittacosis agent. These data are presented in Table 4. Neutralization assays were conducted as described earlier and the calculated $MIPLD_{50}$/ml for the immune and normal ascitic fluids are shown in the 3rd column; the neutralization index is shown in the last column. The neutralization indices for the Borg and 6BC agents were 5.3 and 1.7, respectively, indicating that the neutralizing substance present in the Borg immune ascitic fluid was quite specific.

TABLE 4. SEROLOGICAL SPECIFICITY OF BORG IMMUNE ASCITIC FLUID

Sample	Agent Used in Neutralization Assay	Calculated $MIPLD_{50}$/ml (log_{10})	Log Neutralization Index
Immune ascitic fluid[a/]	Borg	2.1	5.3
Normal ascitic fluid	Borg	7.4	
Immune ascitic fluid	6BC	5.1	1.7
Normal ascitic fluid	6BC	6.8	

a. F-18 (4/13/65).

Table 5 shows the preliminary neutralization data obtained with two different preparations of 6BC immune ascitic fluids. The neutralization index indicated that the neutralizing capacity of the 6BC preparations was much less than had been observed with the Borg fluids. The best 6BC preparation neutralized approximately 3 logs of homologous agent. Since the weakest preparation was used in the cross-neutralization assay, it was not possible to determine the serological specificity of the 6BC immune ascitic fluid. Further studies with these preparations are in progress.

Initially, we were not able to demonstrate neutralization by the intracranial (ic) route; however, as we obtained immune ascitic fluids of greater potency, neutralization became evident by this route. Table 6 shows the results of one of these experiments. The dilutions of the Borg agent used in this experiment ranged from 10^{-5} through 10^{-8}. Because the lower dilutions were omitted, we were unable to calculate the $MIPLD_{50}$; however, these data show that neutralization can be demonstrated by the ic route.

TABLE 5. 6BC IMMUNE ASCITIC FLUID

Sample	Agent Used in Neutralization Assay	Calculated MIPLD$_{50}$ ($\log_{10}$)[a]	Log Neutralization Index
Immune ascitic fluid - 1	6BC	3.4	2.8
Immune ascitic fluid - 2	6BC	5.2	1.0
Normal ascitic fluid	6BC	6.2	
Immune ascitic fluid - 2	Borg	7.3	Neg
Normal ascitic fluid	Borg	7.1	

a. Per 0.5 ml.

TABLE 6. NEUTRALIZATION ASSAY BY INTRACRANIAL ROUTE

Sample	Dilution of Borg Agent ($\log_{10}$)			
	-5	-6	-7	-8
Immune Borg ascitic fluid	5/6[a]	6/6	6/6	6/6
Normal ascitic fluid	1/6	3/6	5/6	6/6

a. Survivors/Totals.

Additional investigations have shown that the neutralizing substance present in the hyperimmune ascitic fluid was not destroyed by heating at 60 C for 1 hour. Also, after the present studies were completed, it was noted that neutralization was complete in less than 30 minutes; therefore, the 4-hour incubation period described earlier could be reduced considerably.

In summary, we have presented a method for the production of immune ascitic fluid in mice for the Borg strain of the human pneumonitis agent, and the 6BC strain of the psittacosis agent. The protective antibodies are easily and quickly obtained and can be made available in large volumes. Indications are that they would serve as suitable typing antisera and perhaps provide material useful for future investigations on the antigenic and immunologic structure of this group of microorganisms.